IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Gregory Collier, et al.	**Examiner:**	Christine J. Saoud
Serial No:	10/039,050	**Art Unit:**	1647
Filed:	December 31, 2001	**Docket:**	12785
For:	NOVEL GENES AND THEIR USE IN THE MODULATION OF OBESITY, DIABETES AND ENERGY IMBALANCE		

Confirmation No.: 2282

Commissioner for Patents
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. §1.131

Sir:

We, Gregory Collier, Paul Z. Zimmet, Kenneth R. Walder, Kelly F. Windmill and Janine S. McMillan, hereby declare that:

1. We are co-inventors named in the above-identified application.

2. We have been advised by our legal representative that U.S. Patent Publication 2002/0055627 to Rosen et al. (" the '627 application") has been cited by the Examiner as a prior art reference under §102(e) in respect to the above-identified application. The '627 application was filed on August 10, 2001, as a continuation of PCT/US00/05883, filed on March 8, 2000, which claims priority from Provisional Application 60/124,270, filed on March 12, 1999.

3. One embodiment disclosed and claimed in the present application is directed to an isolated nucleic acid molecule encoding for a protein which comprises the amino acid sequence set forth in SEQ ID NO: 6, also referred to in the application as "human B55" protein. One such nucleic acid molecule, also disclosed and claimed in the present application, is the nucleic acid molecule having a nucleotide sequence set forth in SEQ ID NO: 5.

4. We isolated the human B55 nucleic acid molecule having the sequence as set forth in SEQ ID NO: 5 in Australia prior to March 12, 1999, the alleged priority date of the '627 application.

5. As evidence of the isolation of the human B55 molecule having SEQ ID NO: 5, we provide herewith Exhibit A. Exhibit A is a copy of the facsimile transmission sent by Professor Greg Collier (a co-inventor) to Dr. Neville McCarthy on a date prior to March 12, 1999. The date has been masked in the preparation of the photocopies contained in Exhibit A. The facsimile transmission was understood to be a confidential communication.

6. The transmission consists of a cover page and six pages of a Progress Report with respect to "Band 55", the designation for B55. The nucleotide sequence of SEQ ID NO: 5 was disclosed at the top of page 3 of the Progress Report, under the heading "Band 55 mRNA HUMAN SEQUENCE".

7. We declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____ Dated: 16/12/05

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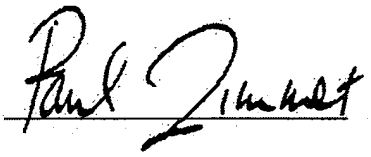

Dated: December 16, 2005

By: _____ Dated: _____

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_____ Dated: _____

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J. McMillan Dated: 16th December, 2005

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FACSIMILE COVER SHEET

Date: DATE MASKED

No. of pages (incl this page): 7

To: Dr Neville McCarthy

Facsimile No: 03 9234 1190

From: Prof Greg Collier

Facsimile No: (03) 5227 2191

Message:

Dear Neville

As discussed please find following a summary of the progress we have made with Band 55.

Cheers

Greg

FAXED

BAND 55

Sequence and Structure

The band 55 mRNA is 1155 nucleotides in length and does not match any known genes in the public database, but has homology with expressed sequence tags (ESTs) from a variety of tissues. The predicted open reading frame results in a protein of 189 amino acids in *Psammomys obesus*. Mouse, rat and human sequences were deduced from ESTs (3 rat, 5 mouse and 8 human sequences were used). The mouse and rat protein were found to be 188 amino acids long and were 91% and 93% homologous to the *Psammomys obesus* sequence, respectively. The human protein was found to be 187 amino acids long and was 82% homologous to the *Psammomys obesus* sequence. There were no nucleotide or amino acid differences found between lean, obese or diabetic *Psammomys obesus*. Band 55 is located on chromosome 15 in humans from 15q26.1 to 15qter and on chromosome 7 in mice.

Band 55 is predicted to have one transmembrane region at residues 37 to 53 with a C-terminal cytoplasmic tail. The tail contains a coiled coil region from amino acids 79 to 117. Coiled coil regions are found predominantly in some structural proteins and in a class of DNA-binding proteins in which the coiled coil region is called a leucine zipper domain. The coiled coil in band 55 is only about 40 residues long, much shorter than the very long coils found in many fibrous proteins such as mysosin and keratin. It also does not appear to be a leucine zipper which are characterized by a leucine every seventh residue. There are 5 leucines, all of which are at *a* or *d* sites but they do not line up down one side of the helix. Coiled coils are found within many other proteins, however, and mediate a wide variety of functions.

A dileucine motif was also found in the cytoplasmic tail. Dileucine motifs have been shown to be involved in trans Golgi sorting, lysosomal targeting and internalization of a number of proteins. The insulin receptor, β2-adrenergic receptor and the glucose transporter GLUT4 all have a dileucine motif which is involved in internalization.

Band 55 has one potential PEST sequence (RPQEEDGPGPSTSSSVTR). Proteins with intracellular half-lives of less than two hours are found to contain regions rich in proline, glutamic acid, serine and threonine (P, E, S and T). These so called PEST regions are generally flanked by clusters of positively charged amino acids. The ProtParam computer program instability index also classified the protein as unstable.

BAND 55 mRNA SEQUENCE

GTCGTTGGTTTCGGCGGCCATGGAGAGCGCAGAGGAGCCTCTGCCCGCGCGGCCGGCGCTGGAGACCGAGG
GCCTGAGGTTCCTGCACGTCACAGTGGGCTCCCTGCTGGCCAGCTATGGCTGGTACGTCCTCTTCAGCTGC
ATCCTTCTCTACATTGTCATCCAGAAGCTCTCCGTCCGATTGAGGGTTTTGAGGCAGAGGCAGCTGGACCA
GGCTGACGCTGTTCTGGAACCTGATGCTGTTGTTAAGCGACAAGAGGCTTTAGCCGCTGCTCGTTTGAGAA
TGCAGGAAGATCTAAATGCCCAAGTTGAAAAGCATAAGGAAAAACTAAGACAGCTTGAAGAAGAAAAAAGG
AGACAGAAGATTGAAATGTGGGACAGCATGCAAGAAGGCAGAAGTTACAGAAGAAATCCAGGAAGGCCTCA
GGAAGAAGATGGTCCTGGACCTTCTACTTCATCATCTGTCACCCGCAAAGGAAAATCTGACAAAAAGCCTT
TGAGGGGAAATGGTTATAACCCTCTGACGGGTGAAGGGGGTGGAACCTGCGCCTGGAGACCTGGACGCAGG
GGCCCATCATCTGGTGGATGAAGCTAAGACCCTTGTTAGTGTCGCTTTGACATTAGCAAGGTGAACCCTTA
ACCCTCAACTCAGTTGCCTTACGCACACTTTCACAGTGACTAGCCAAGGAGAGGTGGGGCTTATTTCCATT
CGTAGCTACCTGTATTCTAAGGGCTTTGGTCAGTGTGAGCTATGGACATTGTCATTAGGTCATATTCTACT
TAGACAACAGTCATTGATTTCATGGCTACTTGCTAGTTGATAGGTTAAAGGCCTCTCGCTGTTTAGCAAAC
TTCATAAAGGAGGCCCAGTGATGATCCTTTGGGGTAGAAGTCCTTGCTGACAGGATGGTCTCTGTGACAGG
ATGCGTTCAATGATGTCTTCCTTATAAATGGTGAGCCCACCAGTGAGGATTACTGATGTGCACAGTTGATG
GGGTTTGCTTCTGTATATTTATTTTTATGTACAGAAATTTGCAAAAAAAAATAAAAAGTAACATTTTTAGC
ATCTTTATTAAACTCAAGGAAATTTCGTTGTGAGCTTGACTTTGTCTATCAGACATTAAACAGCTTTTTAT
CATTAAAAAAAAAAAAAAAAAAAAAAAAAAAA

BAND 55 PROTEIN SEQUENCE (189 aa)

MESAEEPLPARPALETEGLRFLHVTVGSLLASYGWYVLFSCILLYIVIQKLSVRLRVLRQRQLDQADAVLE
PDAVVKRQEALAAARLRMQEDLNAQVEKHKEKLRQLEEEKRRQKIEMWDSMQEGRSYRRNPGRPQEEDGPG
PSTSSSVTRKGKSDKKPLRGNGYNPLTGEGGGTCAWRPGRRGPSSGG

BAND 55 mRNA HUMAN SEQUENCE

CAGGGCTGGGCGGCGGCGGCGGCGGCGGTCATGGAACGCCAAGAGGAGTCTCTGTCCGCGCGGCCGGCCCT
GGAGACCGAGGGGCTGCGCTTCCTGCACACCACGGTGGGCTCCCTGCTGGCCACCTATGGCTGGTACATCG
TCTTCAGCTGCATCCTTCTCTACGTGGTCTTTCAGAAGCTTTCCGCCCGGCTAAGAGCCTTGAGGCAGAGG
CAGCTGGACCGAGCTGCGGCTGCTGTGGAACCTGATGTTGTTGTTAAACGACAAGAAGCTTTAGCAGCTGC
TCGACTGAAAATGCAAGAAGAACTAAATGCGCAAGTTGAAAAGCATAAGGAAAAACTGAAACAACTTGAAG
AAGAAAAAAGGAGACAGAAGATTGAAATGTGGGACAGCATGCAAGAAGGAAAAAGTTACAAAGGAAATGCA
AAGAAGCCCCAGGAGGAAGACAGTCCTGGGCCTTCCACTTCATCTGTCCTGAAACGGAAATCGGACAGAAA
GCCTTTGCGGGGAGGAGGTTATAACCCGTTGTCTGGTGAAGGAGGCGGAGCTTGCTCCTGGAGACCTGGAC
GCAGAGGCCCGTCATCTGGCGGATGAGGCTAAGAATCTTGTTAGTGTCACTTTTGACATTAGCAAGATGAA
CCCTTAACCCTCGATTCAATTGCCTTACGCACGCTTTTCACAGTGACTAGCCAAGGGGAGGTGGGGTTGAT
TTCTGTTCCTAACTACACCTGCATATGTCAGGGCTCCAGTCAGCAAAAGGTATAGATGTTGCCTCTAGGCA
TGAGGTCATTGGTCACATTCTACTTGGAGACAGTGATTGCATTCATTGATTTCATGGTTAATTGCTAGTTG
GTAGGTAAAGGCCTCTAGATGATTAGCAATCTTGATAAAAGAGGCCTAGTAATGTTCTTTTGAGGTTAGAA
ATCCTTGCTGCTAGGACAGTCTCTGTGACAGGTTGCGTTGAATGATGTCTTCCTTATCAATGGTGAGCCCA
CCAGTGAGGATTACTGATGTGGACAGTTGATGGGGTTTGTTTCTGTATATTTATTTTTATGTACAGAACTT
TGTAAAAACGAAACTATTTAAAAAACAAGAATAACATTTTTAGCATCTTTATTCAAGGAGATTTATGGACT
TCAATTTGTCTATCAAACATTAAATAGCTTTTTATTAC

BAND 55 PROTEIN HUMAN SEQUENCE (187 aa)

MERQEESLSARPALETEGLRFLHTTVGSLLATYGWYIVFSCILLYVVFQKLSARLRALRQRQLDRAAAAVE
PDVVVKRQEALAAARLKMQEELNAQVEKHKEKLKQLEEEKRRQKIEMWDSMQEGKSYKGNAKKPQEEDSPG
PSTSSVLKRKSDRKPLRGGGYNPLSGEGG??CSWRPGRRGPSSGG

Suggested that we assume the unknown amino acids at positions 172 and 173 as G and T respectively, based on the sand rat sequence.

Band 55 protein

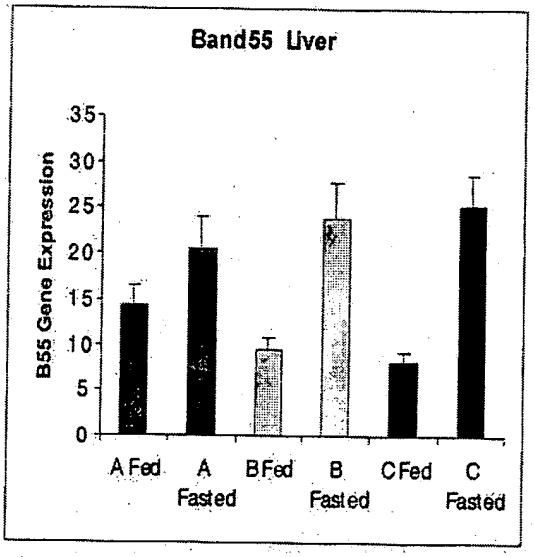
```
                10        20        30        40        50
ISR       MESAEEPLPARPALETEGLRFLHVTVGSLLASYGWYVLFSCILLYIVIQK
MOUSE     -DRD----S-@------S-*----------------I-----------R
RAT            ---S--------S-----------------I----V--------
HUMAN     --RQ--S-S--------------T-------T----IV-------V-F--

                60        70        80        90       100
ISR       LSVRLRALRQRQLDQADAVLEPDAVVKRQEALAAARLRMQEDLNAQVEKH
MOUSE     --L-------------ET-----V-------------------------
RAT       --L-------------E------V-------------------------
HUMAN     --A-----------R-A-AV---V-------------K---E--------

               110       120       130       140       150
ISR       KEKLRQLEEEKRRQKIEMWDSMQEGRSYRRNPGRPQEEDGPGPSTSSSVT
MOUSE     ----------------------------K--S-------------/---I
RAT       ----------------------------K---------------/---I
HUMAN     ----K--------------------#--KG-AKK-----S-----/---L

               160       170       180       189
ISR       RKGKSDKKPLRGNGYNPLTGEGGGTCAWRPGRRGPSSGG
MOUSE     P-----------G-------------S------------
RAT       P-----------G-----
HUMAN     /-R---R-----G-----S----??-S------------
```

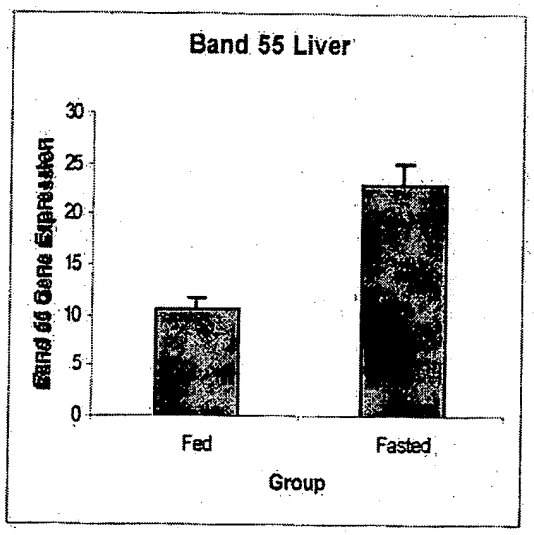
@=R or S
* = R or Q
# = R or K
A = mixture of A and V

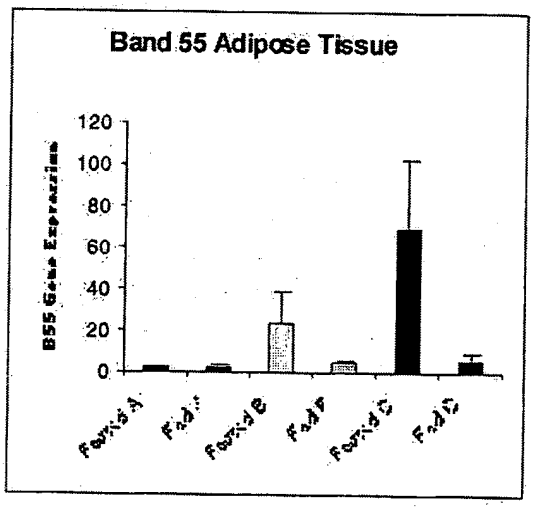
Gene Expression

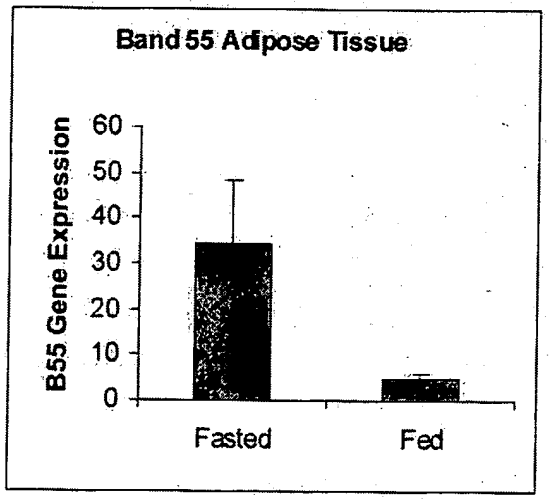
Band 55 gene expression was found to be significantly upregulated in the liver of fasted compared to fed animals ($p<0.0001$). This was evident in groups A, B and C, and the difference appeared more pronounced in obese, diabetic animals. A similar trend was observed in the adipose tissue, with higher levels of expression after fasting ($p<0.05$). This was found in groups B and C only, with the greatest difference in C animals.

In the fed state, there was a significant correlation between liver gene expression and blood triglyceride levels ($p<0.01$).









Cell culture studies

Glucose and insulin effects – HepG2 cells (grown in high glucose media) were treated with different concentrations of insulin (5nM, 50nM and 500nM) for 4 or 24 hours. 4 hours of insulin treatment in high glucose media caused a dose-dependent decrease in band 55 expression. Treatment with 5nM insulin caused a 25% reduction in band 55 expression whilst 50nM and 500nM insulin caused a 42%-43% reduction in expression. The decrease in band 55 expression with insulin treatment was statistically significant at 50nM and 500nM (ANOVA, $p < 0.05$) when compared to the untreated controls. A similar result was observed after 24 hours treatment with insulin (5nM, 50nM, 500nM) in high glucose media. 5nM insulin for 24 hours caused a 23% reduction in band 55 gene expression whilst 50nM and 500nM insulin produced a 62%-63% reduction in expression. Although, band 55 expression was decreased with 24 hours of insulin treatment, it was not statistically significant due to the variability within the control (no insulin) group of cells.

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